STUDIES ON THE ALKALOIDS OF *CEPHALOTAXUS*. III. 4-HYDROXYCEPHALOTAXINE, A NEW ALKALOID FROM *CEPHALOTAXUS FORTUNEI*

GUANG-EN MA[1] and GO-QING SUN

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China

and

MAHMOUD A. ELSOHLY* and CARLTON E. TURNER

School of Pharmacy, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677

ABSTRACT.—Repeated chromatography of an alkaloidal fraction of the ethanolic extract of *Cephalotaxus fortunei* afforded a new alkaloid, mp 135–137°, $C_{18}N_{21}NO_5$. The structure of this alkaloid was shown to be 4-hydroxycephalotaxine (7) by spectral analysis and comparison of the data with those of cephalotaxine (**1a**).

Cephalotaxus fortunei Hook f. is an evergreen tree widely distributed in the southern part of China. The fruits of this plant have long been used in Chinese folk medicine for treatment of tumors. Since 1971, we have studied the alkaloids of this plant and isolated and characterized several alkaloids (1,2) such as cephalotaxine (**1a**), harringtonine (**1b**), homoharringtonine (**1c**), 11-hydroxycephalotaxine (**2**), (+)-acetylcephalotaxine (**3**), 3-epiwilsonine (**4**), cephalofortuneine (**5**) and

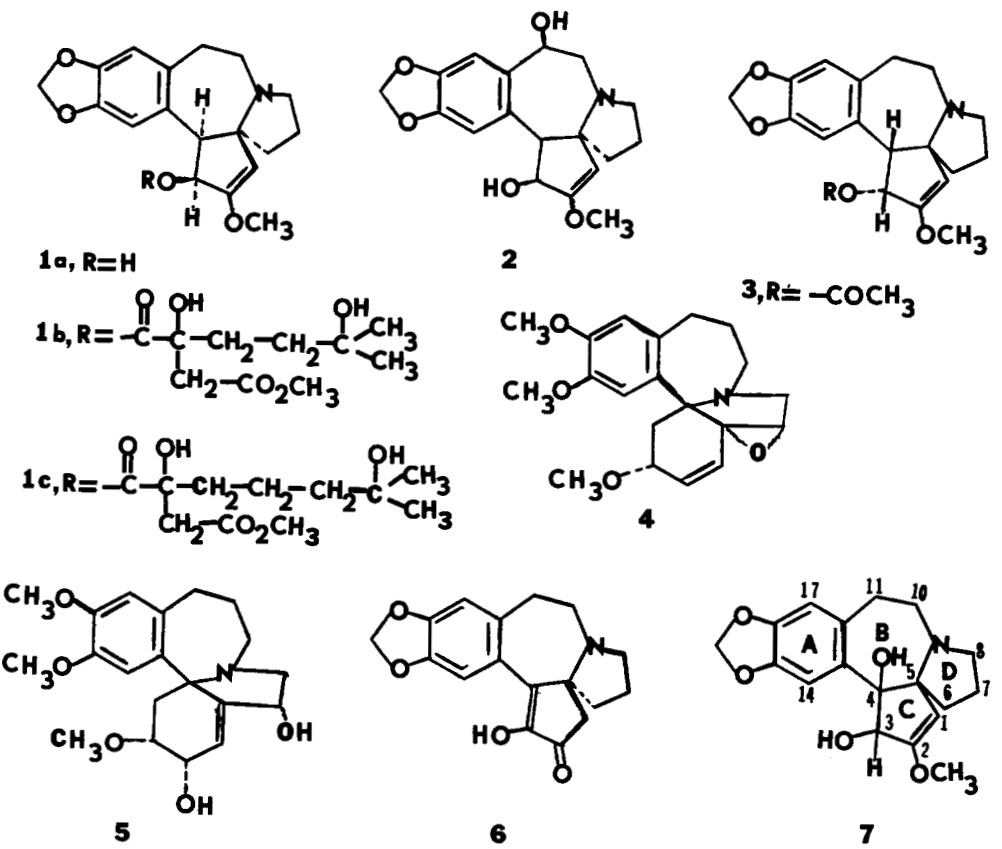

FIGURE 1. *Cephalotaxus* alkaloids.

[1]Currently a visiting scholar at the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.

desmethylcephalotaxinone (**6**), (fig. 1). Harringtonine (**1b**) and homoharringtonine (**1c**), which have been previously reported by Powell *et al.* (3), were shown to have antileukemic activity both in animals and in humans in clinical studies and are used in China for treatment of acute leukemia (4,5). For a review on cephalotaxine esters and their biological activity, see reference 6. Further investigation of the alkaloids of this species has resulted in the isolation of a new alkaloid designated as alkaloid CF–16, which was identified as 4-hydroxy-cephalotaxine.

RESULTS AND DISCUSSION

The isolation of alkaloids from *Cephalotaxus fortunei* was reported earlier (1) and is summarized in chart 1.

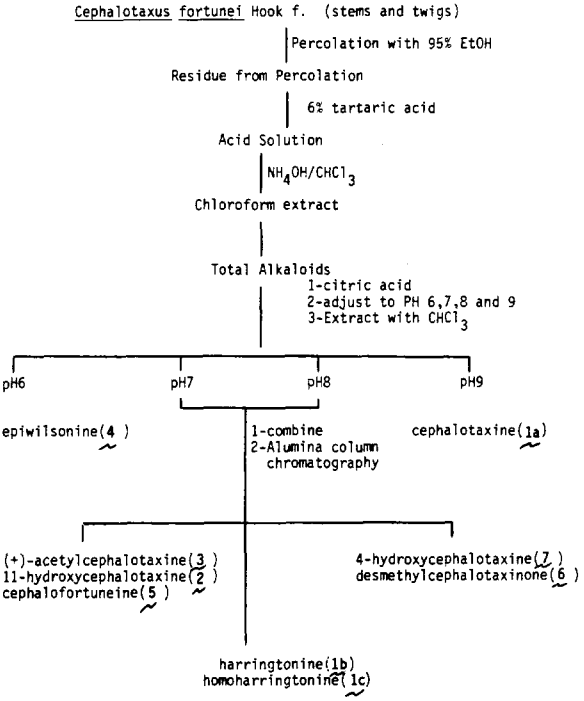

Chart 1. Fractionation scheme of the ethanolic extract of *Cephalotaxus fortunei*.

Rechromatography of the fraction containing alkaloid CF–16 over alumina followed by crystallization from acetone-methanol afforded pale-yellow crystals, mp 135–137°; $[\alpha]^{27}D+120°$ (*c* 0.025, methanol). Spectral analysis of alkaloid CF–16 showed close relationship to cephalotaxine (**1a**). The pmr spectrum showed the following important signals: a two proton singlet at δ 5.84 assigned for a methylenedioxy group, a one proton singlet at δ 4.88 (olefinic), one proton singlet at δ 4.42 for a proton on oxygenated carbon, a three proton singlet at δ 3.60 for a methoxy function, and two aromatic protons at δ 6.54(s) and 7.28(s). In addition two D_2O exchangeable protons were observed between δ 1.80–1.90. A comparison of these data with those of cephalotaxine (table 1) shows close similarity except for the absence of a C4 proton in the spectrum of alkaloid CF–16. The assignment of the signals for C1–H and C17–H was confirmed by NOE experiment. Irradiation at the methoxy resonance (δ 3.60) resulted in an 8% increase in the intensity of the C1–H signal at δ 4.88. Similarly, irradiation of the benzylic protons at C11 at δ 2.10 caused a 13% increase in the intensity of the C17–H at δ 6.54. The mass spectral analysis (table 2) shows a molecular ion at *m/z* 331.1502 for $C_{18}H_{21}NO_5$, which is one oxygen atom more than cephalotaxine. This suggests that alkaloid CF–16 could be a hydroxyderivative of

TABLE 1. Pmr chemical shifts (ppm) of 4-hydroxycephalotaxine and cephalotaxine.

Protons	Cephalotaxine (1b)	4-Hydroxycephalotaxine (7)
C14-H	6.60 (1H, s)	7.28 (1H, s)
C17-H	6.56 (1H, s)	6.54 (1H, s)
OHC_2O	5.81 (2H, s)	5.84 (2H, s)
C1-H	4.85 (1H, s)	4.88 (1H, s)
C3-H	4.66 (1H, d, J=9Hz)	4.42 (1H, s)
C4-H	3.60 (1H, d, J=9Hz)	
C2-OCH_3	3.66 (1H, s)	3.60 (1H, s)

TABLE 2. Significant fragment ions in the mass spectrum of 4-Hydroxycephalotaxine.

Ion	Observed (*m/z*)	Calculated (*m/z*	Empirical formula
a	331.1502 (M^+)	331.1494	$C_{18}H_{21}NO_5$
b	314.1340	314.1349	$C_{18}H_{20}NO_4$
c	300.1250	300.1249	$C_{17}H_{18}NO_4$
d	298.1105	298.1103	$C_{17}H_{16}NO_4$
e	282.1181	282.1188	$C_{17}H_{16}NO_3$
f	216.1062	216.1058	$C_{13}H_{14}NO_2$

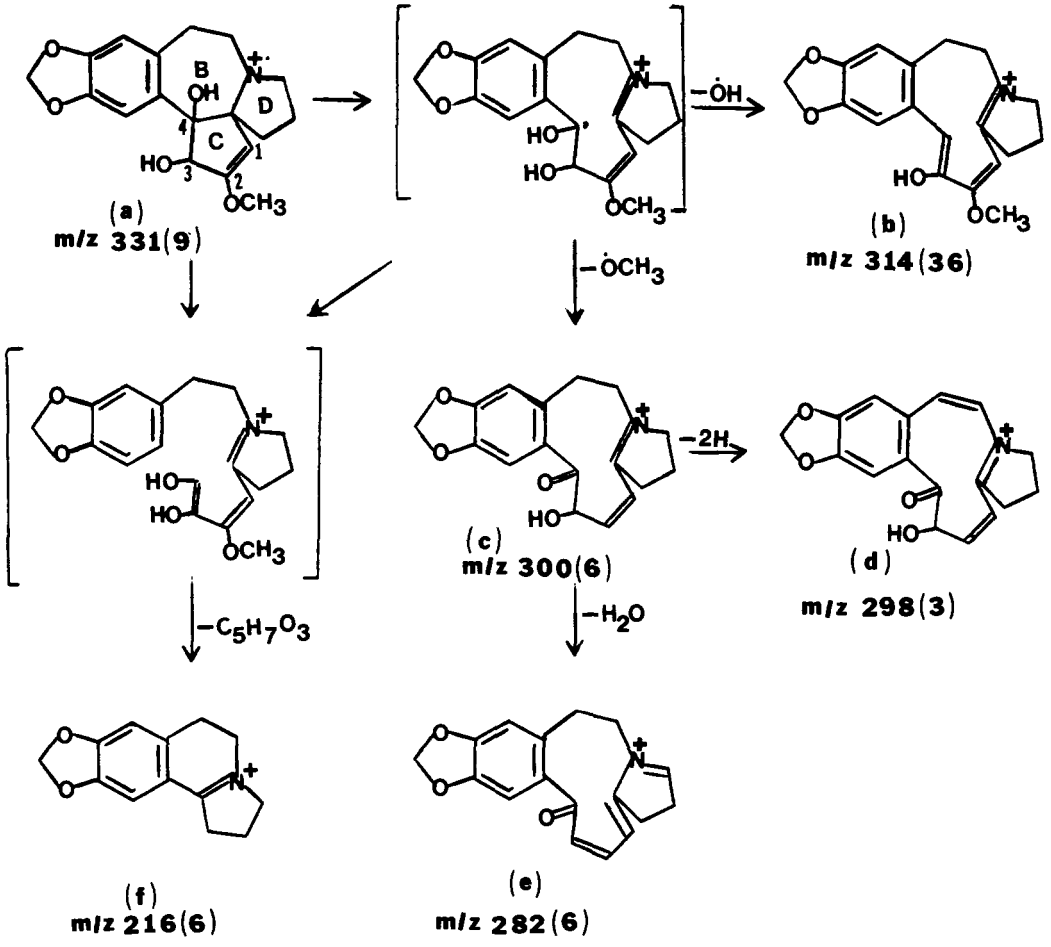

FIGURE 2. Mass spectral fragments of 4-Hydroxycephalotaxine.

cephalotaxine. The absence of a C4 proton in the pmr spectrum of alkaloid CF–16 and the presence of two D_2O exchangeable protons suggest that the extra hydroxy group must be located on C4. This is substantiated by the fact that C3–H shows a sharp singlet in the pmr spectrum of alkaloid CF–16 as opposed to a doublet ($J=9$Hz) in the spectrum of cephalotaxine. These data are thus consistent with the assignment of structure 7, 4-hydroxycephalotaxine, to alkaloid CF–16. The proposed structure was further supported by studying its mass fragmentation. Figure 2 shows structure assignments for the major fragments in the ms spectrum of 4-hydroxycephalotaxine. The presence of fragments b-f and, particularly, fragment f is supportive of the location of the two hydroxy groups in ring C (C–3) and C/B (C–4). This is the first reported isolation of this alkaloid from nature. The stereochemistry of 4-hydroxycephalotaxine (7) is still undetermined. However, the fact that the proton on C14 is much more deshielded (7.28 ppm) than the corresponding proton in other cephalotaxine-type alkaloids (7) could be explained if C4–OH was *trans* to the C3–OH of cephalotaxine. In this structure the proton of the C4–OH is in close proximity to the C14 proton.

EXPERIMENTAL[2]

PLANT MATERIAL.—The stems and twigs of *Cephalotaxus fortunei* Hook f. used in this investigation were collected during the spring of 1974 at the foot of Huangshan Mountain, Anhwei Province, China. Herbarium specimens are deposited in the Shanghai Institute of Materia Medica, Chinese Academy of Science, People's Republic of China.

EXTRACTION AND FRACTIONATION.—The powdered plant material of *C. fortunei* was percolated with 95% ethanol (1:8 w/v) at room temperature. The ethanol extract was concentrated in vacuum, and water was gradually added until all ethanol was evaporated. The aqueous solution (1/10 of the plant material w/v) was acidified with tartaric acid to pH 3–4 and was kept in the refrigerator over night. The insoluble material was separated and washed with 6% tartaric acid, and all washings were added to the original supernatant.

The combined supernatant and washings were made alkaline by addition of ammonium hydroxide and extracted with chloroform until the reacidified solution no longer gave reaction for alkaloids with Mayer's reagent. The combined chloroform layers, upon evaporation, afforded a crude alkaloidal residue which was dissolved in 5% citric acid and filtered. The filtrate was then adjusted to pH 6,7,8 and 9 with ammonium hydroxide and extracted with chloroform at each pH. The procedure for treatment of each alkaloidal fraction has been previously described in detail (1) and shown in chart 1.

ISOLATION OF 4-HYDROXYCEPHALOTAXINE.—The alkaloid fractions obtained at pH7 and pH8 were dissolved in chloroform and chromatographed over neutral alumina and eluted with chloroform followed by 1% to 10% methanol-chloroform mixtures. Fractions eluted with 5% methanol-chloroform were evaporated to give a yellowish residue. Rechromatography over alumina and crystallization from methanol afforded pale-yellow crystals, mp 135–137°, $[\alpha]^{27}D+120°$ (*c* 0.025, Methanol); uv: λmax (MeOH) (log ε) 240 (4.09) and 290 (4.06) nm; ir: νmax (KBr) 3400, 3200 (OH), 1650 (c=c) 1610, 1590, 1580 (aromatic ring) and 930 cm^{-1} (OCH_2O); pmr ($CDCl_3$): δ4.88 (1H, s), 3.60 (3H, s), 4.42 (1H, s), 5.84 (2H, s), 6.54 (1H, s), 7.28 (1H, s) and 1.80–1.90 (2H, br, s, exchangeable with D_2O); ms: *m/z* 331 (9%, M^+), 314 (36), 300 (6), 298 (3), 282 (6), 216 (6) and 109 (100).

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[2]The melting points were taken on a Kofler apparatus and were uncorrected. The optical rotations were measured on a Perkin-Elmer-141 polarimeter. The ultraviolet spectra were determined on a Sp-1800 model recording spectrometer. The infrared spectra were determined on a SP–100 spectrometer in KBr pellets. Proton nuclear magnetic resonance spectra were recorded in $CDCl_3$ with a JEOL–PS–100 instrument with HMDS as the internal standard and reported in δ (ppm) units. Mass spectra were obtained with a MAT–711 mass spectrometer and a Finnigan-3200 GC/MS/DS instrument.

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